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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application : Mary Jo Ondrechen et al.
Application No. : 10/678,756
Filed : October 2, 2003
Confirmation No. : 6620
For : COMPUTATIONAL METHOD FOR PREDICTING PROTEIN INTERACTION SITES
Examiner : Larry D. Riggs II
Attorney's Docket : NU-593XX

TC Art Unit: 1631

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I hereby certify that this correspondence is being sent via facsimile to Examiner Larry D. Riggs II, TC Art Unit 1631, Fax No. (571) 273 8300, on Aug. 15, 2008.

By: Holliday C. Heine
Holliday C. Heine, Ph.D.
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* *

DECLARATION OF DR. MARY JO ONDRECHEN UNDER 37 C.F.R. §1.132

VIA FACSIMILE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Mary Jo Ondrechen hereby declare that:

1. I hold the position of Professor of Chemistry and Chemical Biology at Northeastern University in Boston, Massachusetts.

2. My areas of expertise include chemistry and genomics.

3. I am a co-inventor of the subject matter described and claimed in the above-identified patent application.

4. I have read and understood the Office Action of the Examiner dated May 20, 2008, rejecting claims 1-3, 5 and 11-13 of the currently pending claims as being anticipated by Beroza et al.

5. In my opinion, as one of ordinary skill in the art, there are a number of ways in which the Examiner has mischaracterized the teachings of Beroza et al. The Beroza paper (Beroza, P., et al., *Electrostatic calculations of amino acid titration and electron transfer, Q-AQB-->QAQ-B, in the reaction center*. Biophys J, 1995. **68**(6):2233-50) is about one very specialized protein, namely the photosynthetic reaction center. The purpose of the Beroza paper is to explain apparent observed discrepancies in experimental data pertaining to the photosynthetic reaction center, which is a highly unusual assembly, not typical of an enzyme. In particular, it is unusual in that it has multiple reactive areas and that it has bound co-factor molecules, including the quinone molecules, Q_A and Q_B, that take part in the electron transfer reactions.

6. The perturbed shapes of some of the computed titration curves for the photosynthetic reaction center, as reported in the Beroza paper, do indeed explain the apparent discrepancies in the previously reported experimental data for one residue in particular, the GluL212. However, the information in the Beroza paper would not lead one of ordinary skill to the conclusion that one could *predict* active sites from observed anomalies in the

shapes of computed titration curves, for reasons such as the following.

7. The Beroza paper talks about two types of unusual titration behavior, namely 1) a shifted pK_a and 2) an anomalous titration curve shape. Shifted pK_as are indeed common in residues at enzyme active sites, but computed pK_as are not very good *predictors* of active sites, as I have discussed in recent articles (Wei, Y., J. Ko, L. Murga, and M.J. Ondrechen, *Selective prediction of Interaction sites in protein structures with THEMATICS*. BMC Bioinformatics, 2007. **8**:119; and Tong, W., R.J. Williams, Y. Wei, L.F. Murga, J. Ko, and M.J. Ondrechen, *Enhanced performance in prediction of protein active sites with THEMATICS and support vector machines*. Protein Sci., 2008. **17**:333-341). Attempting to identify active sites using residues with computed pK_a values that are shifted far from their normal values leads to too many false positives and false negatives and thus to poor predictions.

8. The claimed invention deals specifically with anomalies of the second type, unusual titration curve shapes. With regard to unusual titration curve shapes, the Beroza paper talks about two quinone molecules, Q_A and Q_B, that are bound to the protein assembly in the photosynthetic reaction center. It also talks about a cluster of residues near each of the two quinone molecules. First, I shall discuss the cluster of residues near Q_A. In Figure 3A, the Beroza paper shows a perturbed titration curve shape for one residue, ArgM247. Figure 3A shows a more typical curve shape (sigmoidal) for GluL104. Curve shapes are not

shown for the other residues in Figure 3A because they are shifted out of range. Thus, from the information in Figure 3A one finds only one residue with a perturbed curve shape. This one residue does not constitute a cluster, as recited in our claim.

9. For the residues near Q_B, shown in Figure 3B, the Beroza paper does indicate that these computed theoretical titration curves have disturbed shapes for this particular local area of the photosynthetic reaction center and that these residues with perturbed titration curves are located in a physical cluster. However, there is absolutely no information given to indicate that this is true in any other active part of the reaction center protein or in the active site in any other protein other than the photosynthetic reaction center. In other words, there is nothing to indicate that this phenomenon could be general. If a cluster of residues with perturbed titration curves is observed in this one part of the photosynthetic reaction center, but no such phenomena are reported for the other reactive parts of the photosynthetic reaction center, how could this indicate to one of ordinary skill that he or she could use this information to *identify* reactive areas inside a protein, in the manner claimed in the instant application?

10. It would not have been obvious to one of ordinary skill from the teachings of the Beroza paper that the reported unusual shapes of the calculated titration curves would be observable in any other protein. In fact, because the photosynthetic reaction center is so special, one of ordinary skill might instead conclude that the reported titration behavior is *actually unique*

to one particular, local area inside the photosynthetic reaction center.

11. The Beroza paper also indicates that the active residues in the photosynthetic reaction center have shifted pK_as. Based on the information provided in Figures 3A and 3B, it might appear that shifted pK_as are a better predictor of reactive zones than the disturbed curve shapes. We have found this not to be the case, as I have discussed above.

12. In order to be useful to identify active sites, an observed phenomenon has to be highly prevalent in active sites and also uncommon elsewhere in the protein structure. There is nothing in the Beroza paper to suggest either of these.

Attorney Docket No: NU-593XX
Inventor(s): Mary Jo Ondrechen et al.
Application No.: 10/678,756
TC Art Unit: 1631/Examiner: Larry D. Riggs II
OA Date: 05/20/2008

I hereby declare that all statements made herein on personal knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 14th day of August, 2008.

By Mary Jo Ondrechen
Mary Jo Ondrechen

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